



# Trace Metal Residues in Swimming Warrior Crab *Callinectes bellicosus*: A Consumption Risk

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This study was carried out in the Navachiste coastal lagoon, Mexico, surrounded by intensive agricultural and aquaculture activities that cause environmental pollution by the deposition of trace metal residues in the sediments of this coastal lagoon. The trace metals are bioaccumulated by benthic organisms such as the blue swimming warrior crab, *Callinectes bellicosus*, which inhabits this lagoon and is consumed by humans. Ninety-five *C. bellicosus* edible tissue samples were collected (April 2014–January 2015). The extraction procedure of the trace metals in edible tissue samples was carried out by acid digestion with nitric acid. Based on the Environmental Protection Agency (EPA) of the United States, two indices were used to measure health risk: the estimated daily intake (EDI) and the target hazard quotient (THQ). The hazard index (HI) was used to calculate the probability of adverse carcinogenic risk and the target hazard quotient per sample (MHI) to calculate the probability of developing a carcinogenic or non-carcinogenic risk. The analysis of variance (ANOVA) showed significant differences among trace metal concentrations ( $p < 0.01$ ), but all trace metal concentrations in the edible tissues of *C. bellicosus* were higher than the maximum residual limits (MRLs). The highest EDI was for Zn, Fe, and Cu, showing that the consumption of these crabs might represent health risks. The THQ >1 was for Ni, Zn, Cd, and Cu, and the HI = 16 revealed the risk of *C. bellicosus* for high-level consumers. The MHI showed that 98% of samples presented a THQ >1, implying a high rate of bioaccumulation of trace metals by the crabs independent of the sampling site in the NAV. The presence of trace metals in the edible tissue of crabs reflects contamination by trace metals, and the indices results mean that the NAV lagoon is constantly polluted with trace metal residues by neighboring agriculture and aquaculture activities. These trace metal residues are being bioaccumulated in the edible tissues of *C. bellicosus* due to its feeding habits, resulting in a health risk if its consumption is high, including carcinogenic and non-carcinogenic risks.

**Keywords:** seafood, heavy metal, trace elements, Navachiste, environmental pollution

**Abbreviations:** NBay, Navachiste Bay, Sinaloa, Mexico; NAV, Navachiste coastal lagoon system; PCA, principal components analysis.

## INTRODUCTION

The risk of exposure by humans to trace metals has increased significantly in industrial and agricultural regions. Coastal lagoons are some of the areas most impacted by the discharges of these pollutant residues (Pan and Wang, 2012; Meena et al., 2017; Yang et al., 2018). One of these impacted regions is the coastal lagoon system of Navachiste (NAV) located in the southeastern part of the Gulf of California. It is a semiclosed coastal lagoon in a semiarid and subtropical area with sand barrier islands and significant extensions of mangrove areas, columnar cacti, dry deciduous forests, wetlands, and shrublands. NAV is surrounded by the largest agricultural region in Mexico and more than 9,000 ha of shrimp aquaculture farms (Carrasquilla-Henao et al., 2013) that are constantly discharging pollutant residues, such as trace metals, which enter the coastal lagoons (Pan and Wang, 2012; Meena et al., 2017; Yang et al., 2018). Metal residues are found naturally in soils and sediments, but enrichment has been related to agricultural and aquaculture practices (Jalali and Hemati, 2013). NAV is being impacted by the residues drained from the agricultural Guasave Valley (Martínez-Valenzuela et al., 2009) and by the large quantities of fish excrement, uneaten feed, antibiotics, fungicides, and antifouling agents released by the aquaculture activities (Mateo-Sagasta et al., 2018).

Previous studies carried out in NAV have described how trace metal concentrations in its sediments have become bioavailable to marine species (Páez-Osuna and Osuna-Martínez, 2015), including those of commercial interest (Páez-Osuna and Osuna-Martínez, 2015; Góngora-Gómez et al., 2018; Delgado-Alvarez et al., 2019). In the NAV, one of the most important artisanal fisheries is the blue swimming warrior crab (*Callinectes bellicosus*) which is exported to international markets at up to 13,000 tons year<sup>-1</sup> (Ortega-Lizárraga et al., 2020).

Due to the commercial importance of *C. bellicosus*, the increased bioavailability of trace metals in sediments of NAV, and the ability of this crab to induce bioturbation of sediments because of its omnivorous feeding habit that resuspends the metal residues, which, in turn, become bioaccumulated in its tissues, *C. bellicosus* represents a human health risk to consumers. In this sense, monitoring these metal traces in the edible tissue of *C. bellicosus* is a significant concern for health risks. Thus, the main objective of this study was to determine the trace metal residue content in the edible tissue of the *C. bellicosus* crab to evaluate the carcinogenic and non-carcinogenic risks posed by the consumption of this species.

## MATERIAL AND METHODS

The NAV encloses three lagoons (San Ignacio, Macapule, and Navachiste) located in the southeastern Gulf of California (25° 27'59"N and 108° 50'24"W). Samples were collected during spring, summer, autumn, and winter, with crab traps from April 2014 to January 2015 (Figure 1). The sampling sites were selected based on two characteristics: their proximity to discharge drainages from agricultural, urban, and shrimp farming

areas and locations not influenced by any drainage effluents. Water parameters (pH, temperature, salinity, and dissolved oxygen) were recorded with a HANNA® HI-9828 multiparameter (HANNA Instruments, Italy). The samples were packed in polyethylene bags to avoid contamination with trace metal residues of other packing materials, like aluminum foils, and cold stored in a 40-L cooler to delay the oxidation of organic matter in sediments until freezing in the Environmental Contamination Laboratory of the CIIDIR-IPN, Sinaloa Unit.

Ninety-five samples of edible tissues of the crab species *C. bellicosus* were processed. The trace metals were extracted by acid digestion with nitric acid based on the Breder method for the extraction of metal residues in sediments by atomic absorption spectrometric methods and for silicate sediments (Breder, 1982). The muscle tissues of each sample were dehydrated; subsequently, 0.5 g per sample was supplemented with 5 ml of HNO<sub>3</sub> (65%) and placed for 4 h in an aluminum heating block with precise temperature control to dissolve the organic matter strongly adhered to sediments. A similar procedure was performed with 0.5 g of the sediment that was tested with 5 ml of a 1:3 HCl-HNO<sub>3</sub> mix. This mixture does not decompose the sediment, and high recovery of the metallic elements is achieved, and the extractions are precise in determining metals such as As, Cu, Cr, Hg, Mn, Ni, Pb, V, and Zn. After digestion, the samples were cooled to room temperature, gauged to 50 ml with deionized water, and transferred to a graduated polypropylene Falcon® tube. A GBC AVANTA®, USA, atomic absorption spectrometer, with a programmable air-acetylene flame and hollow cathode lamps, was used to detect and determine trace metals. The precision of the instruments and techniques was adjusted using the TORT-2 lobster hepatopancrea reference material, NRC-CNRC®, which was treated like the samples. Six calibration curves (0.125, 0.25, 0.5, 1, 2, and 4 mg L<sup>-1</sup>) were prepared using the standard certified PerkinElmer® solution (1000 mg L<sup>-1</sup>). The limit of detection (LOD) for each metal was calculated using the following equation (INMETRO 2016) (Eq. 1):

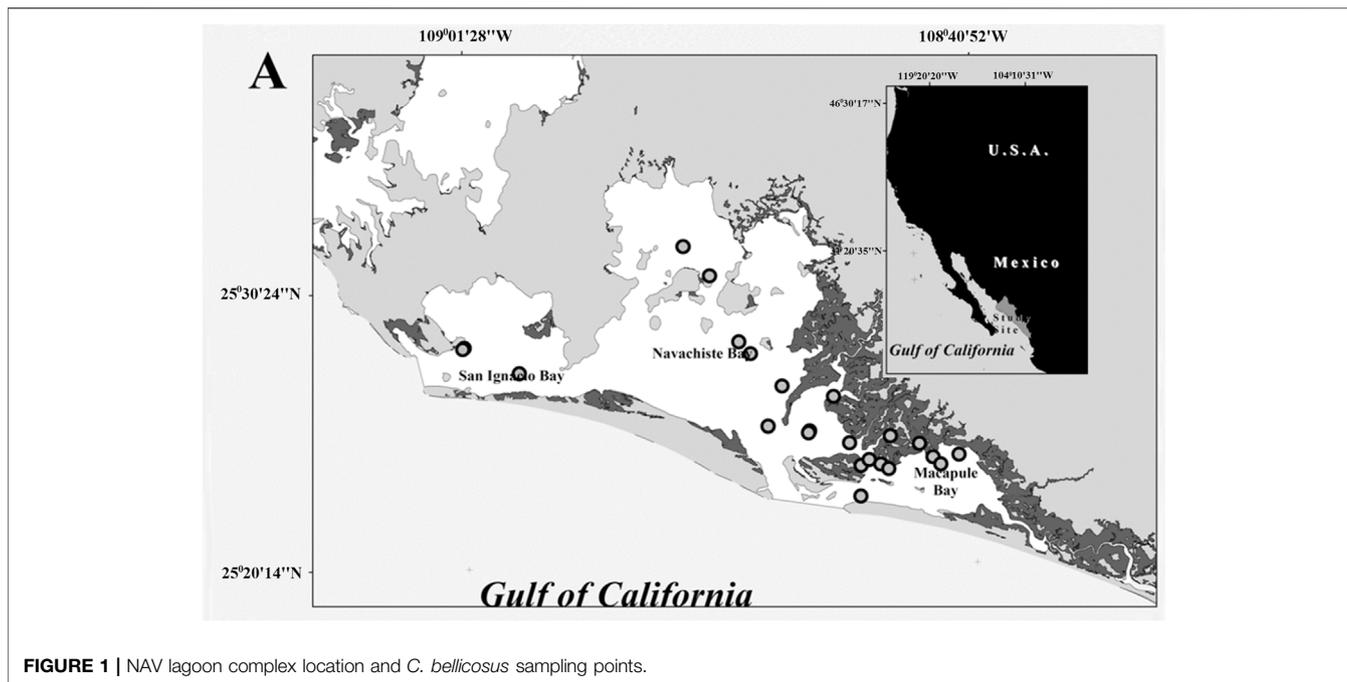
$$\text{LOD} = 10 \times S_{bl} \quad (1)$$

where  $S_{bl}$  is the standard deviation of 10 control blanks analyzed, in the present report, and the LOD was 0.0158 mg CaCO<sub>3</sub> L<sup>-1</sup>.

As recommended by the U.S. Environmental Protection Agency of the (USEPA), three indices were used to measure health risks: estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI). In this study, we propose the use of the total metal THQ per sample (MTHQ) index. The estimated daily intake (EDI) (μg kg<sup>-1</sup> day<sup>-1</sup>) is the exposure to a chemical residue or consumption of a nutrient and was calculated considering the resulting metal concentration, crab consumption, and mean body weight chart for U.S. adults, following the next equation (Eq. 2) (U.S. Environmental Protection Agency, 2013):

$$\text{EDI} = \frac{C_m \times F_{IR}}{BW} \quad (2)$$

Here,  $C_m$  = metal concentration of the sample (mg kg<sup>-1</sup> ww);  $F_{IR}$  = seafood ingestion rate in the United States (0.227 g pers<sup>-1</sup> day<sup>-1</sup>)



**FIGURE 1** | NAV lagoon complex location and *C. bellicosus* sampling points.

(USEPA, 2000); and BW is the average body weight of adults, which was determined at 70 kg.

The target hazard quotient (THQ) (U.S. Environmental Protection Agency, 2005) is the health risk posed by the swimming warrior blue crab and was calculated based on the following equation (U.S. Environmental Protection Agency, 2018) (Eq. 3):

$$THQ = \frac{EF \times ED \times CS \times MC}{BW \times AT \times R_{FD}} \times 10^{-3} \quad (3)$$

where *EF* = exposure frequency (350 days year<sup>-1</sup>), *ED* = human exposure duration—70 years (average lifetime), *CS* = seafood meal size in the United States for average crab consumers (0.227 g pers<sup>-1</sup> day<sup>-1</sup>), *MC* = metal concentration in one crab edible portion (mg kg<sup>-1</sup> ww), *BW* = adult body weight (70 kg), *AT* = average time (*ED* × 365 days/year), and *R<sub>FD</sub>* = oral reference dose (mg kg<sup>-1</sup> of body weight per day) (USEPA, 1989; 2000). *R<sub>FD</sub>* is an estimate of daily oral exposure to a toxic substance during a lifetime for a human population (Bress, 2009). *R<sub>FD</sub>* reported for Cd and Pb were 0.0003 and 0.00005 mg kg<sup>-1</sup> day<sup>-1</sup>, respectively (Hassett-Sipple et al., 1997); for Cu, Fe, Mn, Ni, and Zn, they were 0.04, 0.7, 0.024, 0.00026, and 0.3 mg kg<sup>-1</sup> day<sup>-1</sup>, respectively (U.S. Environmental Protection Agency, 2018).

The hazard index (HI) is the probability of developing a carcinogenic or non-carcinogenic risk and was calculated to evaluate the risk of all trace metals. It corresponds to the total of calculated THQ trace metal concentrations in all samples and indicates the ratio between exposure and the reference dose as follows (Jović and Stanković, 2014) (Eq. 4):

$$HI = \sum_{i=1}^n THQ_i \quad (4)$$

where THQ<sub>*i*</sub> = target hazard quotient of individual trace metals and *n* = number of examined trace metals (in the present study,

*n* = 7). HI, ≤1 means a non-probability of adverse carcinogenic risk; HI >1 indicates a probability of adverse effects, and HI ≥10 suggests the presence of a high likelihood of chronic risk (Lei et al., 2015).

In the present study, we calculated the target hazard quotient per sample (MHI) to determine the carcinogenic and non-carcinogenic risk per sample as follows (Eq. 5):

$$MHI = \left( \sum_{i=1}^n M_i THQ_i \right) \quad (5)$$

where M<sub>*i*</sub>THQ<sub>*i*</sub> = is the sum of the target hazard quotient of each trace metal per sample *n* (in the present study, *n* = 95). As in the THQ index, values of MHI ≤1 mean a non-probability of adverse carcinogenic risk, MHI >1 indicates a probability of adverse effects. MHI ≥10 suggests the presence of a high likelihood of chronic risk.

The data were statistically analyzed with SAS® (v. 9) and Statistica® (ver. 7) and log<sub>10</sub> transformed for a Kolmogorov–Smirnov (*p* > 0.05, α = 0.05) test and ANOVA (*p* < 0.05, α = 0.05); when significant differences were detected a post hoc Tukey HSD (Vasavada, 2014) test was applied. Pearson’s correlation test (*p* < 0.05) was used for trace metal concentration and weight, size, and physicochemical seawater parameters. A Spearman’s correlation, followed by principal components analysis (PCA) (*p* < 0.05), was performed among seasons and physicochemical seawater parameters and trace metal concentrations.

## RESULTS AND DISCUSSION

The weight of crabs ranged from 102 to 386 g ww (238.2 ± 76.7 g ww), the width and length of shell oscillated from 10.5 to 16.5 cm,

and 5.5–9 cm, respectively. The trace metal average concentrations ( $\text{mg kg}^{-1}$ ) detected in the 95 edible samples of *C. bellicosus* were  $1.77 \pm 2.98$  (Cd),  $64.23 \pm 30.94$  (Cu),  $65.18 \pm 35.35$  (Fe),  $6.0 \pm 4.04$  (Mn),  $6.34 \pm 3.38$  (Ni),  $5.27 \pm 2.97$  (Pb), and  $184.37 \pm 76.21$  (Zn). The ranges of trace metal concentrations were for Cd 0.073–11.034, Ni 0.18–12.63, Pb 0.88–13.63, Cu 18.15–190.77, Fe 24.57–257.64, Mn 0.024–14.82, and Zn 77.29–571.20. The concentration sequence of trace metals was  $\text{Zn} > \text{Fe} > \text{Cu} > \text{Mn} \approx \text{Ni} > \text{Pb} > \text{Cd}$ . Higher concentrations of Zn, Fe, and Cu could be attributed to agricultural residues. This activity uses formulants that contain glyphosate-based herbicides and other pesticides usually rich in one or more of these metals (Defarge et al., 2018). This suggests that discharges from the surrounding agricultural or aquaculture activities are currently present at the NAV (Martínez-López et al., 2017) increasing after irrigation activities (Páez-Osuna and Osuna-Martínez, 2015). Discharged trace metal residues have been trapped in the NAV sediments, as previously reported (Montes et al., 2012) and bioaccumulated by *Callinectes* species. As previously reported, the presence of traces of metals in the edible tissue of crabs reflects contamination by metal residues in estuarine ecosystems (Anandkumar et al., 2019; Truchet et al., 2020), as occurring in NAV. Metals (Hg, Zn, Cd, Cu, and others), metalloids (As), and radioisotope residues are degraded very slowly due to their long geochemical cycle and the increase in disturbances and acceleration of metal residues produced by anthropogenic activities that may be accumulated in the sediments, where they can stay for years (Wuana and Okieimen, 2011), leading to the persistence of toxicants in the environment (Peng et al., 2009). After that, they become bioavailable and are absorbed by marine biota after the irrigation season that begins in October (presowing) and ends in January (Sifuentes et al., 2016). However, more studies must be done to correlate the bioavailability of trace metal concentrations with sedimentation rates, pollutants, and organic matter concentrations in the effluents.

In the NAV system, anthropic source metals are added and dispersed in the lagoon. These anthropic compounds contain Cd and Pb impurities, which increase their content in the soil after fertilization application (Wuana and Okieimen, 2011). Local reports indicate that in phosphate fertilizers, the average content of Pb and Cd is 10.9 and 10.4  $\text{mg kg}^{-1}$ , respectively; for nitrogen fertilizers, Pb and Cd contents are 4.7 mg and 2.03  $\text{mg kg}^{-1}$ , respectively (Romano Casas et al., 2019). The primary source of phosphorus fertilizers is phosphorite rocks, mainly consisting of apatite naturally enriched by lanthanides such as Cu, Ni, and Zn (Boumaza et al., 2021). These fertilizers, widely used in Sinaloa, are an essential source of diverse elements in soils. In the region, Cd concentrations are low during the spring, with a slight increase in late summer (Romano Casas et al., 2019). These concentrations could be associated with the indiscriminate use/application of fertilizers that, consequently, drain their residues into the NAV and with primary productivity in the water column, the content of organic matter, clay minerals, and hydrothermal events during its formation.

In the present study, the highest concentrations of metals in sediments and tissues of *C. bellicosus* were found in April, 2 months after the irrigation season, and in sites nearby or in front of the effluents of wastewater from agriculture or aquaculture activities (sites 2, 3, 4, and 5; **Figure 2**). It has been observed that an increase in trace metal concentrations begins in the spring (dry) and decreases during the summer (wet) and winter (dry), 2–3 months after agricultural or aquaculture wastewater discharges. During the sedimentation process that can take months, trace metal residues are precipitated to the sediments (Saleh, 2021), and their composition varies by resuspension, biogeochemical interactions with sourcing areas, sediment resuspension transport, depositional rates, and diagenesis processes (Spagnoli and Bergamini, 1997; Spagnoli et al., 2021). Griboff et al. (2020) found correlations between some trace metals and the dry seasons, resembling the results found in this work. The accumulation of metal residues in sediments of an aquatic system contributes to their dispersion and bioavailability to marine biota. These dispersions and bioavailability in the NAV increase with the presowing irrigation from October to January in the Guasave valley (Sifuentes et al., 2016).

The presence of trace metals in the edible tissue of biota, and sediments from the NAV have been previously reported (Orduna-Rojas and Longoria-Espinoza, 2006; Reyes-Montiel, 2013; Aguilar-Gonzalez et al., 2014; Granados-Galván et al., 2015; Páez-Osuna and Osuna-Martínez, 2015). Trace metals have an affinity with organic matter, which is incorporated into the marine sediments of coastal lagoons, such as NAV. In the sediments, these trace metals become bioavailable, and bioaccumulation of metals in *C. bellicosus* could be related to the feeding habits of the species, the rates of absorption and depuration, the environmental conditions, the bioavailability of the metals, the fine texture of the sediments,  $\text{CaCO}_3$  content, and organic matter content (Jerome and Chukwuka, 2016; Álvaro et al., 2016; Chuan et al., 2017; Genç and Yilmaz, 2017; Çoğun et al., 2017; Annabi et al., 2018; Baki et al., 2018; Durmus et al., 2018; Saber et al., 2018; Hao et al., 2019; Cruz et al., 2021). *C. bellicosus* is an omnivorous species that induces bioturbation of sediments by mixing and resuspending the organic matter associated with the sediment, improving the bioavailability of trace metals (Andrade et al., 2020; Truchet et al., 2020), which are ingested and bioaccumulated in different tissues of this crab.

In invertebrates, the molting stage occurs as a growth process. It is a critical moment in the detoxification of bioaccumulated metals (Anandkumar et al., 2019) that depends mainly on the presence of metallothioneins. Metallothioneins play a role in detoxifying trace metals in estuarine crabs that can translocate and bioaccumulate in the tissues (Truchet et al., 2020). However, the concentrations of these proteins are related to changes in natural factors, such as salinity, weight, or sex (Legras et al., 2000). Indeed, for *C. bellicosus*, it is recommended to carry out studies regarding the concentration of these molecules and perform a correlation with the physiochemical seawater parameters to elucidate whether trace metal concentrations in *C. bellicosus* tissues are

**TABLE 1 |** Average metal content ( $\text{mg}^{-1} \text{kg}^{-1}$ ) in muscle tissue of different crab species.

Element	<i>C. bellicosus</i> (this study)	<i>P. marmoratus</i> <sup>a</sup>	<i>P. segnis</i> <sup>b</sup>	<i>C. sapidus</i>	<i>C. amnicola</i> <sup>f</sup>	<i>P. sanguinolentus</i>	<i>T. crenata</i>	<i>M. victor</i>	<i>E. verrucosa</i>	<i>Scylla</i> spp.
Cu	64.23 ± 30.94	172.91 ± 7.88	206.45 ± 71.88	<b>9.26 ± 0.53<sup>c</sup></b> <b>18.214 ± 2.60<sup>d</sup></b> <b>2.8 ± 0.21<sup>e</sup></b>	<b>9.48–12.76</b>	<b>17.205 ± 0.53<sup>g</sup></b>	<b>30.735 ± 0.36<sup>g</sup></b>	<b>11.4 ± 0.44<sup>g</sup></b>	<b>42.2 ± 4.8<sup>h</sup></b>	65.8 <sup>i</sup>
Cd	1.77 ± 2.98	<b>1.22 ± 1.13</b>	<b>0.21 ± 0.03</b>	<b>1.23 ± 0.19<sup>c</sup></b> <b>0.161 ± 0.24<sup>d</sup></b> <b>0.313 ± 0.048 SE<sup>e</sup></b>	<b>0.16–0.46</b>	19.48 ± 0.06 <sup>g</sup>	12.1 ± 0.29 <sup>g</sup>	8.03 ± 0.29 <sup>g</sup>		<b>0.17<sup>i</sup></b>
Fe	65.18 ± 35.35	658.33 ± 0.1	85.05 ± 18.58	120.64 ± 0.81 <sup>e</sup>		<b>29.56 ± 1.71<sup>g</sup></b>	168.62 ± 1.68 <sup>g</sup>	69.9 ± 2.5 <sup>g</sup>	81.68 ± 5.32 <sup>h</sup>	
Ni	6.34 ± 3.38	<b>5.56 ± 0.80</b>		12.02 ± 0.29 <sup>e</sup>					<b>2.13 ± 0.19<sup>h</sup></b>	
Mn	6.00 ± 4.04	36.42 ± 11.05	<b>0.41 ± 0.08</b>			89.706 ± 1.26 <sup>g</sup>	10.147 ± 0.2 <sup>g</sup>	6.5 ± 3.48 <sup>g</sup>	39 ± 2.9 <sup>h</sup>	
Pb	5.27 ± 2.97	<b>0.38</b>	<b>0.4 ± 0.17</b>	<b>2.66 ± 0.23<sup>c</sup></b> <b>1.208 ± 0.13<sup>d</sup></b>	<b>1.48 ± 3.17</b>	<b>&lt; 0.06<sup>g</sup></b>	<b>&lt; 0.06<sup>g</sup></b>	<b>&lt; 0.06<sup>g</sup></b>		<b>0.20<sup>i</sup></b>
Zn	184.37 ± 76.21	<b>146.19 ± 6.1</b>	590.04 ± 196.9	<b>28.71 ± 2.23<sup>c</sup></b> <b>43.98 ± 3.44<sup>d</sup></b> <b>11.197 ± 0.77<sup>e</sup></b>	<b>2.21–3.65<sup>f</sup></b> <b>16.71 ± 0.77</b>	<b>47.37 ± 0.37<sup>g</sup></b>	<b>61.921 ± 0.43<sup>g</sup></b>	<b>22.6 ± 0.2<sup>g</sup></b>	284.85 ± 12.9 <sup>h</sup>	<b>175<sup>i</sup></b>

Bold data indicate lower trace metal concentrations than those detected in the present study.

<sup>a</sup>Álvarez et al. (2016).

<sup>b</sup>Annabi et al. (2018).

<sup>c</sup>Çoğun et al. (2017).

<sup>d</sup>Gençand Yılmaz (2017).

<sup>e</sup>Saber et al. (2018).

<sup>f</sup>Jerome and Chukwuka (2016).

<sup>g</sup>Baki et al. (2018).

<sup>h</sup>Dumus et al. (2018).

<sup>i</sup>Chuan et al. (2017).

affected. The crab *C. bellicosus* showed higher Cu, Pb, and Zn traces than other estuarine crabs (Table 1). Higher trace metal concentrations in tissues indicate a more significant human disturbance of aquatic systems (Chuan et al., 2017; Genç and Yılmaz, 2017; Baki et al., 2018; Saber et al., 2018). In this sense, the agriculture and aquaculture activities and the increased number of sailing vessels and artisanal outboard fishing in the NAV (Carrasquilla-Henao et al., 2013; Aguilar-Gonzalez et al., 2014) are increasing the bioavailability of trace metals. This correlation of bioavailability and human pollution sources has been previously described. Hamed and Emará (2006) reported high Cu, Zn, Pb, Cd, Cr, Ni, Fe, and Mn in *Patella caerulea* tissue in the Suez Canal. Bazzi (2014) determined that in sediments and marine organisms of the Gulf of Chabahar, the highest values of Zn, Pb, and Cu. In both studies, as in the present, a correlation was found between trace metal concentrations in organisms and human activities, such as shipping, marine transportation, fisheries, and drainage. This higher concentration of Pb in *C. bellicosus* could be explained by the potential use of glyphosate-based pesticides in the region

(Balderrama-Carmona et al., 2020), which includes Pb in their formulation (Defarge et al., 2018), and its bioavailability could be increased by the constant wastewater discharges from urban and agricultural areas into the NAV (Álvarez et al., 2016). Zn has been reported to have a high affinity for organic carbon in sediments (Cyriac et al., 2021) and is an essential component of the reproductive coenzymes in the genus *Callinectes* (Çoğun et al., 2017). In this report, the concentration of Zn and the higher concentrations of Cu confirm the relevance of these elements in the metabolism of estuarine crabs (Anandkumar et al., 2019) like *C. bellicosus*. In addition, the high concentration of Zn and Cu (and the other trace metals) could also be attributed to the high phosphorus, urea, and TSS concentrations that have been reported in the area from April to November drained by agriculture and shrimp aquaculture (Martínez-López et al., 2017; Góngora-Gómez et al., 2018).

Among seasons, Spearman’s correlation revealed no significant differences ( $p < 0.05$ ) between trace metals and pH in the spring, salinity, and temperature in the winter (Table 2).

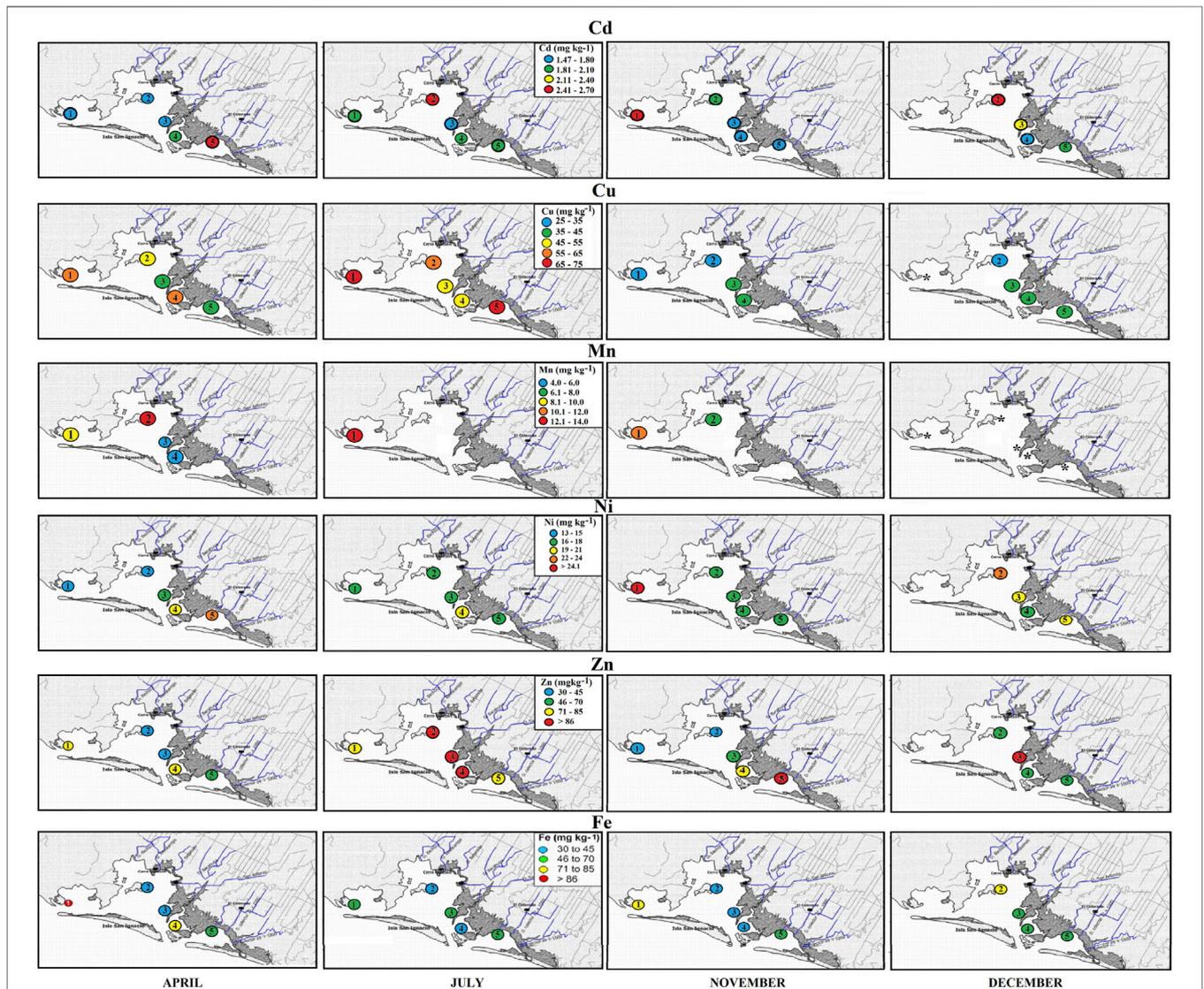
**TABLE 2 |** Pearson’s correlation results of the trace metal concentration in the edible tissue of *C. bellicosus* and the seawater physicochemical parameters of salinity (‰), pH, conductivity ( $\sigma$ ), and temperature (T°C).

Trace metal	‰	pH	$\sigma$	T°C
Cu	-0.1	-0.2	-0.3	0.2
Fe	0.1	-0.1	0.1	-0.1
Mn	0.5	0.1	0.4	-0.2
Zn	0.1	0.0	0.0	-0.1
Cd	0.2	-0.1	-0.9	-0.1
Ni	0.4	-0.2	0.3	-0.1
Pb	-0.1	0.1	0.1	0.3

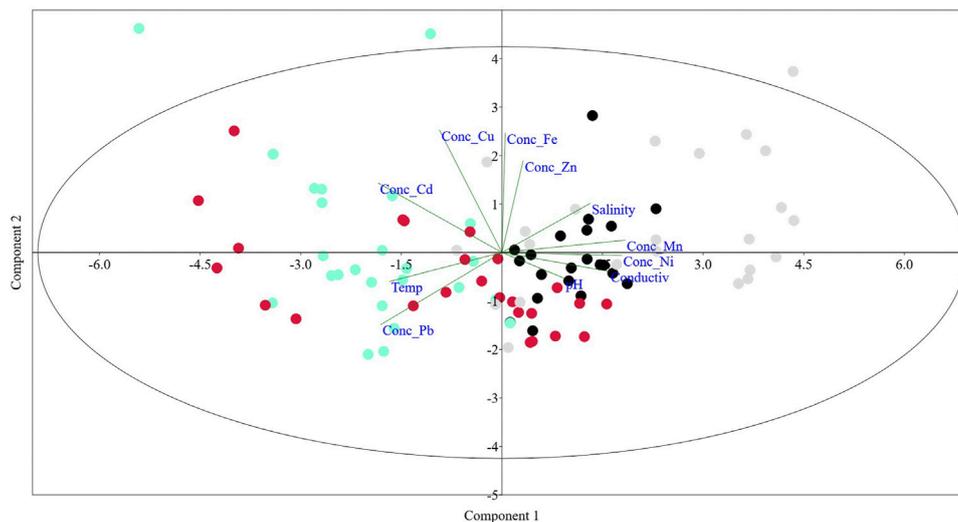
Principal components analysis showed that Mn concentration, conductivity ( $\sigma$ ), and salinity (S‰) were negatively correlated. Summer Pb concentrations showed a negative correlation with temperature (Figure 3). Associations of Mn and Ni were revealed

with conductivity, pH, and salinity in the spring and late autumn and between Pb and temperature in the summer. About weight and trace metal concentrations, a significantly low mean negative correlation ( $p < 0.01$ ) was determined between Zn and weight ( $r = -0.4$ ). The rest of the trace metals showed a non-significant correlation with weight. After the PCA per season ( $p < 0.05$ ), the spring Ni concentrations were high and low negative with  $s$  and pH, respectively. The first two principal components of the multivariate analysis explained 95% of the variance (Figure 2).

Metal concentrations in the edible tissue of *C. bellicosus* from the NAV revealed the presence of trace metals that could pose a risk if higher concentrations and portions are consumed. ANOVA showed significant differences among trace metal concentrations ( $p < 0.01$ ). At a consumption rate of  $0.227 \text{ g}^{-1} \text{ day}^{-1}$ , the average of all trace metal concentrations in the present study was higher than the



**FIGURE 2 |** Average trace metal concentration in the edible tissue of *C. bellicosus* from the collecting sites in the NAV complex, Mexico.



**FIGURE 3** | PCA correlation between water physicochemical parameters and trace metals in the edible tissue of *C. bellicosus* from the NAV complex, Mexico.

maximum residual limits (MRLs) delineated by the European Union (EC, 2008), and the United States Food and Drug Administration (National Shellfish Sanitation Program, 2007). The MRLs (in  $\text{mg kg}^{-1} \text{ dw}$ ) in edible tissues of crustaceans for the EU and the United States are, respectively, for Cd of 0.5 and 3.0; for Ni of 2.8 and 70; and for Pb of 0.5 and 1.5; whereas for Cu, Fe, Mn, and Zn, no records of allowable limits were found. For sediment reference, the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life reference (Canadian Council of Ministers of the Environment, 1999) were used. Fe, Mn, and Zn were abundant elements and presented the following order of concentration:  $\text{Fe} > \text{Mn} > \text{Zn} > \text{Pb} > \text{Ni} > \text{Cu}$ . The primary source of the first element's contribution is weathering of the rocks in the drainage basin. A local report by the Consejo de Recursos Minerales (1991) indicates mineral deposits of Au, Ag, Zn, Pb, Cu, and some of Fe, Ni, Co, Bi, and ferrous deposits constituted by  $\text{Fe}_3\text{O}_4$  (72% of Fe). Iron is present in sediments as iron oxyhydroxides that influence the release of other elements in response to pH changes, affecting the adsorption or desorption of other metals (Queiroz et al., 2021). Correlation coefficients ( $r^2$ ) indicated positive values mainly between Fe and Zn (0.8), Fe and Mn (0.6), Mn and Ni (0.70), and Mn and Cu (0.8), indicating the influence of Fe and Mn oxides on the release of elements. Fe and Mn oxides exert control on the adsorption or coprecipitation of elements in sediments (Turner, 2000) in the lagoon system. These properties during seasonal rains, irrigation in agricultural fields, water exchange in aquaculture farms, and others probably contribute to the flow of these inorganic and organic components in the system, thus modifying the natural conditions and bioaccumulation in organisms.

The enrichment factor (EF) and the geoaccumulation index (Igeo) data of ISQG, the continental crust content (Taylor,

1964), preindustrial levels, and Fe as a normalizing element (Salomons and Förstner, 2012) were used as references (Appendix 1). The geochemical index indicated Pb as uncontaminating to moderately contaminating in January (2017) and Cd as moderately contaminating in April (2016). On the other hand, the enrichment factor presented a similar condition to that of Cd, revealing a severe enrichment of sediments, and Pb as moderately severe to moderately enriched (Table 3). The normalized data of the other elements indicated a lower enrichment and related them with natural sources in the system.

Regarding the risk assessment, the mean EDI values ( $\mu\text{g kg}^{-1} \text{ bw d}^{-1}$ ) of the selected metals ranged from 0.005 to 0.537 in the following sequence:  $\text{Zn} > \text{Fe} > \text{Cu} > \text{Ni} > \text{Mn} > \text{Pb} > \text{Cd}$ . The highest EDI was for Zn, Fe, and Cu (0.54, 0.19, and  $0.19 \mu\text{g kg}^{-1} \text{ day}^{-1}$ , respectively); Zn, Cu, and Cd were above the acceptable daily intake consumption. In contrast, Ni and Mn were similar to reference values (FAO and WHO, 2013), at a rate of  $0.227 \text{ g}^{-1} \text{ day}^{-1}$ , and represent a low concentration for the portion established for consumption of trace metals in the *C. bellicosus* edible tissue ( $\text{EDI} < 1$ ). The average THQ ranged between 0.03 and 6.32. Zn, Cu, and Cd were the trace metals with a  $\text{THQ} > 1$  (7.68, 4.74, and 1.76, respectively), turning these ratios into an exposure risk to trace metals in the edible tissue of the blue swimming warrior crab. The values of  $\text{HI} > 1$  represent a potential exposure to trace metals and adverse effects (Jerome and Chukwuka, 2016; Genç and Yilmaz, 2017; Baki et al., 2018). In the present study, the  $\text{HI} = 16.11$  showed a potential exposure to trace metals in the edible tissue of *C. bellicosus* (Table 4). The MHI for each sample showed that 93 samples (98%) exhibited an  $\text{MHI} > 1$ , indicating a potential carcinogenic or non-carcinogenic health risk of *C. bellicosus* edible tissue consumption (Figure 4).

**TABLE 3 |** Enrichment factor and the geoaccumulation index of trace metals in sediment samples of the Navachiste coastal lagoon system, Mexico.

	Cu	Fe (%)	Mn	Zn	Cd	Ni	Pb
April-16	7.0 ± 2.3	1.6 ± 0.2%	228.2 ± 73.6	50.2 ± 54.4	0.97 ± 0.49	10.0 ± 3.7	9.0 ± 4.2
Jan-17	2.33 ± 1.6	1.8 ± 0.3%	286.1 ± 47.0	50.9 ± 9.3	—	12.5 ± 1.6	24.6 ± 8.4
April-17	6.2 ± 2.7	1.94 ± 0.25%	295.5 ± 55.6	48.35 ± 7.6	—	6.6 ± 2.6	9.9
Bazzi (2014) Inv	21.85–46.8	14.2–53.5	43.2–84.4	16.2–43.1	0.4–0.8	11.7–26.4	13.9–28.2
Bazzi (2014) Ver	10.97–54.76	12.8–52.1	46.9–89.1	18.8–40.1	0.2–0.5	8.3–28.7	10.7–25.6
Laguna Unare, Venezuela (Marquez)	41.1	1.56%	516.4	127.5	1.51	52.4	29
ISQG <sup>a</sup>	18.7	—	—	124	0.7	—	30.2
SQS2	390	—	—	410	5.1	—	450
TEL2	18.7	—	—	124	0.6	15.9	30.2
PEL <sup>a</sup>	108	—	—	271	4.2	42.8	112
ERL2	34	—	—	150	1.2	20.9	46.7
ERM2	270	—	—	410	9.6	51.6	218
Continental crust (Taylor, 1964)	55	5.6%	950	70	0.2	75	12.5
Sadiq (1992) <sup>b</sup> Ref	<10	—	—	<110	<1	<10	<5
Salomons and Forstner (1984) <sup>c</sup>	45	47000	600	95	0.2	68	20

<sup>a</sup>Interim marine sediments quality guidelines (Canadian sediment quality guidelines for the protection of aquatic life) (ISQG, Interim sediment quality guideline; PEL, Probable effect level).

<sup>b</sup>Non-contaminated sediments Marquez et al. (2008) TEL.

TEL2, threshold effect levels. Concentrations below TEL are not associated with any adverse biological effect. Between TEL and PEL, an adverse biological effect can occur occasionally and frequently above PEL. SQS, quality standards of marine sediments. The quality criterion corresponding to sediments neither related with adverse effects on biological resources, including acute and chronic, nor significant risks for human health (WAC, 1995; cited in Fuentes-Hernandez et al., 2019). ERL, low effects interval; ERM, moderate effect interval.

<sup>c</sup>Preindustrial data, from Salomons & Forstner (2012).

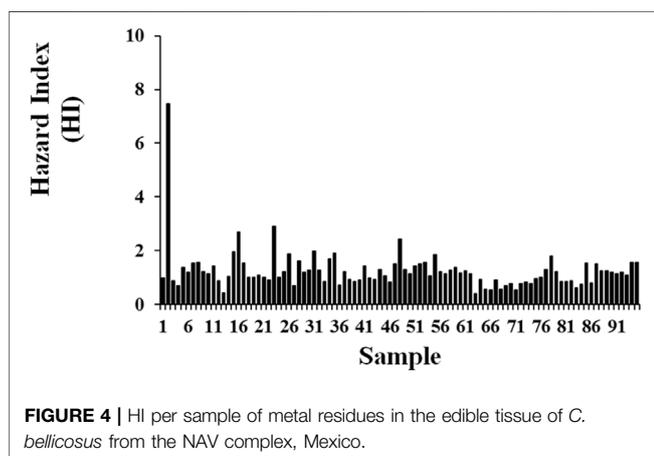
**TABLE 4 |** Average concentration of trace metals, reference dose (RfD), non-carcinogenic health risk (THQ), and hazard risk (HI) in the edible tissue of *C. bellicosus* from the Navachiste coastal lagoon system in Mexico.

Trace metal	Concentration (mg kg <sup>-1</sup> ) M ± SD	RfD (mg kg <sup>-1</sup> day <sup>-1</sup> )	(THQ)	EDI
Cu	64.23 ± 30.94	0.04 <sup>a</sup>	5.29	0.18723
Cd	1.77 ± 2.98	0.001 <sup>b</sup>	5.13	0.00535
Fe	65.18 ± 35.35	0.7 <sup>a</sup>	0.30	0.18999
Mn	6.00 ± 4.04	0.024 <sup>a</sup>	0.75	0.01790
Ni	6.34 ± 3.38	0.02 <sup>a</sup>	1.01	0.01847
Pb	5.27 ± 2.97	0.5 <sup>c</sup>	0.03	0.01502
Zn	184.37 ± 76.21	0.3 <sup>a</sup>	2.15	0.53741
Hazard Index (HI)	14.74	∑ THQ	10.72	

<sup>a</sup>Hassett-Sipple et al. (1997).

<sup>b</sup>U.S. Environmental Protection Agency (2018).

<sup>c</sup>Limit of lead content in white edible tissues of crustaceans (European Commission, 2014).



**FIGURE 4 |** HI per sample of metal residues in the edible tissue of *C. bellicosus* from the NAV complex, Mexico.

## CONCLUSION

The metal residue concentrations in the edible tissue of *C. bellicosus* from the NAV complex were above the maximum allowed metal concentrations in crabs for consumption. Consumption of the edible tissue of *C. bellicosus* from the NAV reveals a risk hazard, including carcinogenic or non-carcinogenic risks. The constant drains from the agricultural Guasave Valley and aquaculture activities after irrigation or wastewater drainage maintain trace metal bioavailability and uptake by the lagoon biota, including the *C. bellicosus* crab species. Pollution from human activities has been reported for a long time. Findings from the present study confirm the lack of strategies to reduce or avoid the discharge of these pollutants into the lagoon or the use of banned pesticides and fertilizers, whose residues are being discharged into the NAV lagoon complex.

According to the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and THQ per sample (MHI), the consumption of *C. bellicosus* edible tissue represents a health risk at a rate of  $0.227 \text{ g}^{-1} \text{ day}^{-1}$  consumption. This risk would be intensified if the consumption rate increases above  $1.2 \text{ g}^{-1} \text{ day}^{-1}$  pers<sup>-1</sup>.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

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## AUTHOR CONTRIBUTIONS

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